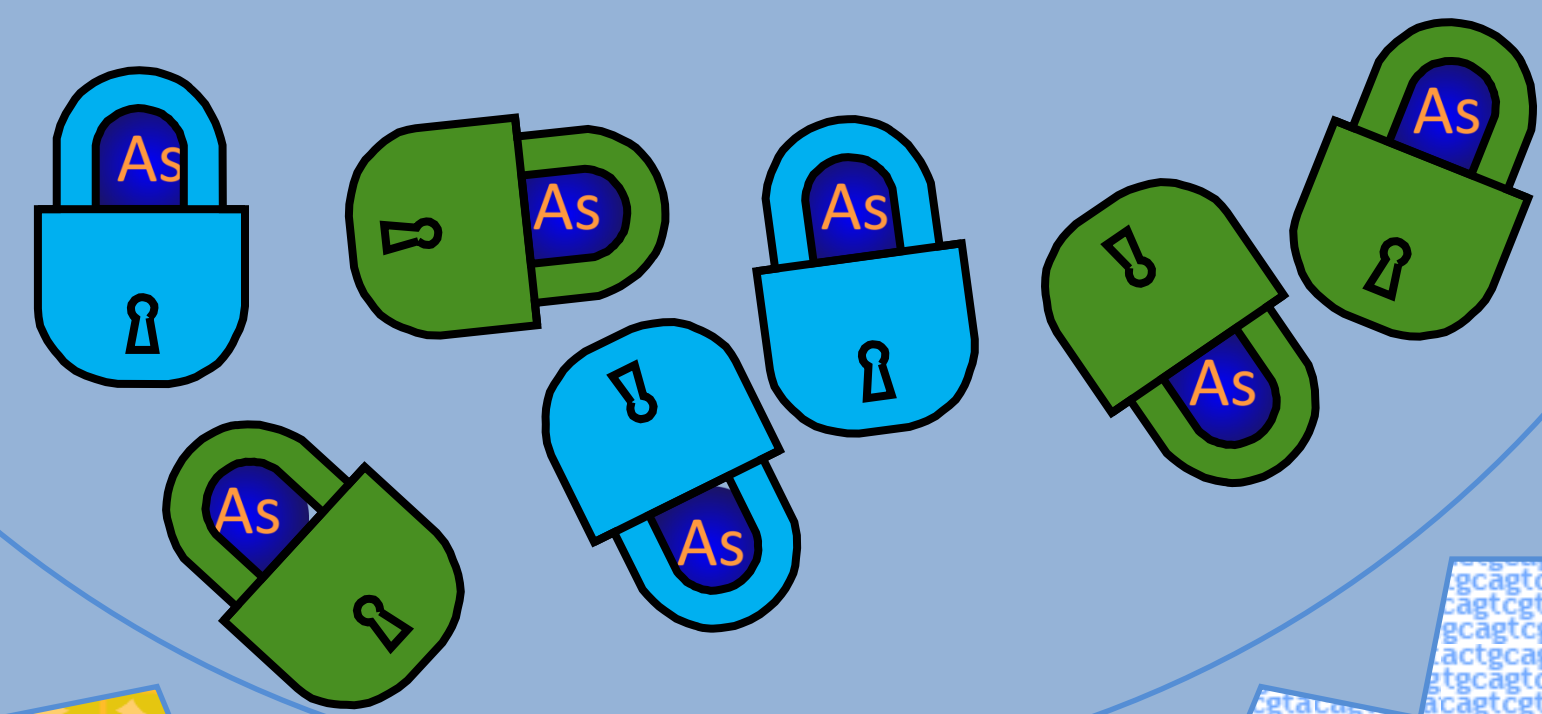# Arsenic Sponge

Problem

Millions of people around the world are affected by ground-water contaminated by arsenic. Existing treatment options are too expensive for the majority of those affected. Therefore we are developing a bioremediation tool using *Escherichia coli* to sequester and bind arsenic. Natural and synthetic peptides are employed to bind the toxic ions and a pump knockout prevents efflux. Measurement of growth capacity of the engineered strain in arsenic containing media and quantitative analysis of arsenic sequestration using HPLC is presented. A simple, well-implemented system for biosequestration of arsenic may become part of a solution to a problem denying access to clean drinking water for many.

Extended consumption of arsenic-contaminated water leads to severe health problems including skin, lung and bladder cancer.

Idea

E. Coli have an endogenous pathway to deal with arsenic toxicity. It reduces and pumps out arsenic cations. To implement a biosequestration solution in this chassis we need to eliminate this efflux of arsenic.

Accumulating arsenic in the cell would quickly lead to cell death and lysis, therefore we need a sequestration mechanism. Something needs to bind the arsenic and prevent it from interfering with cellular processes.
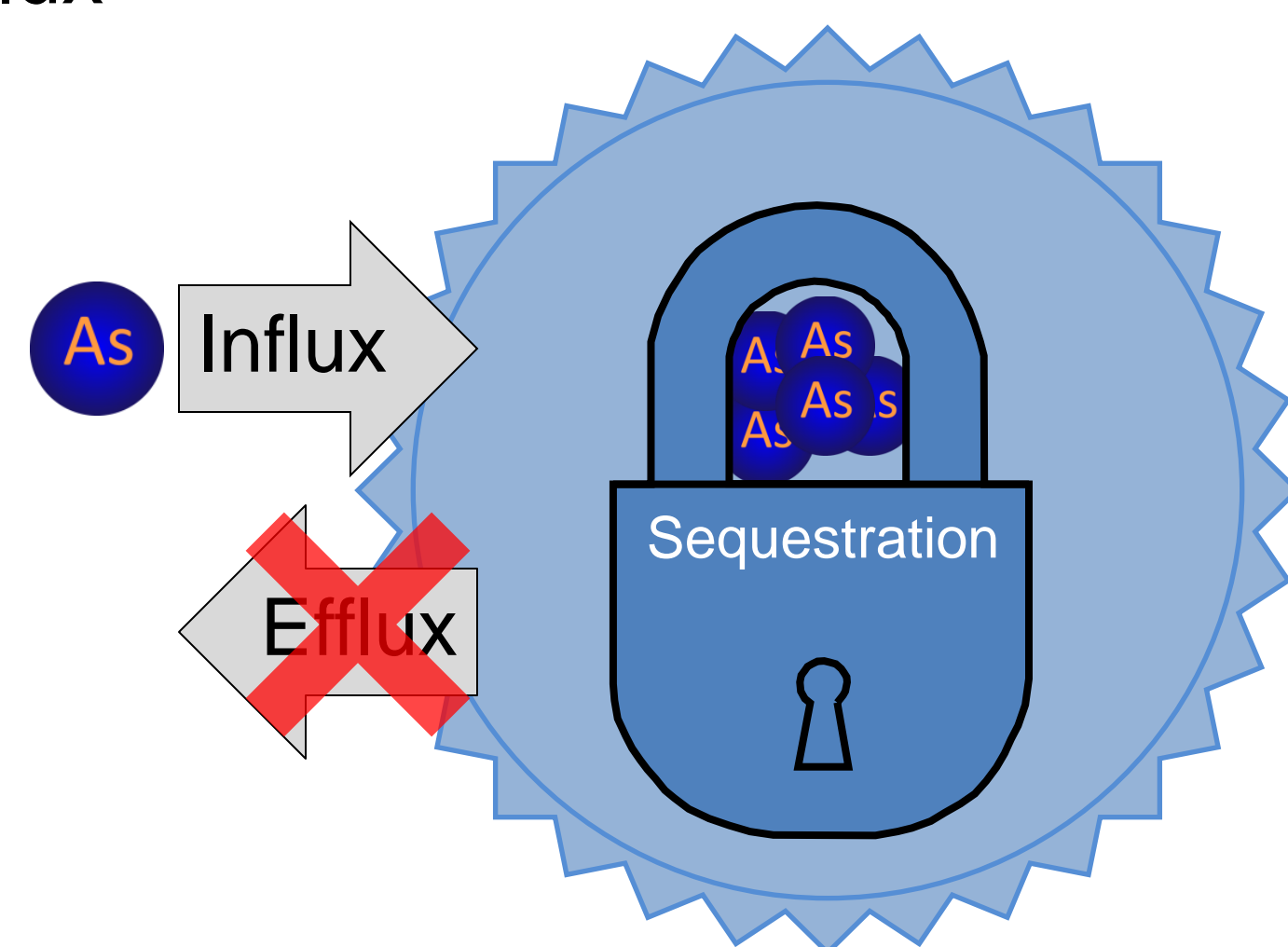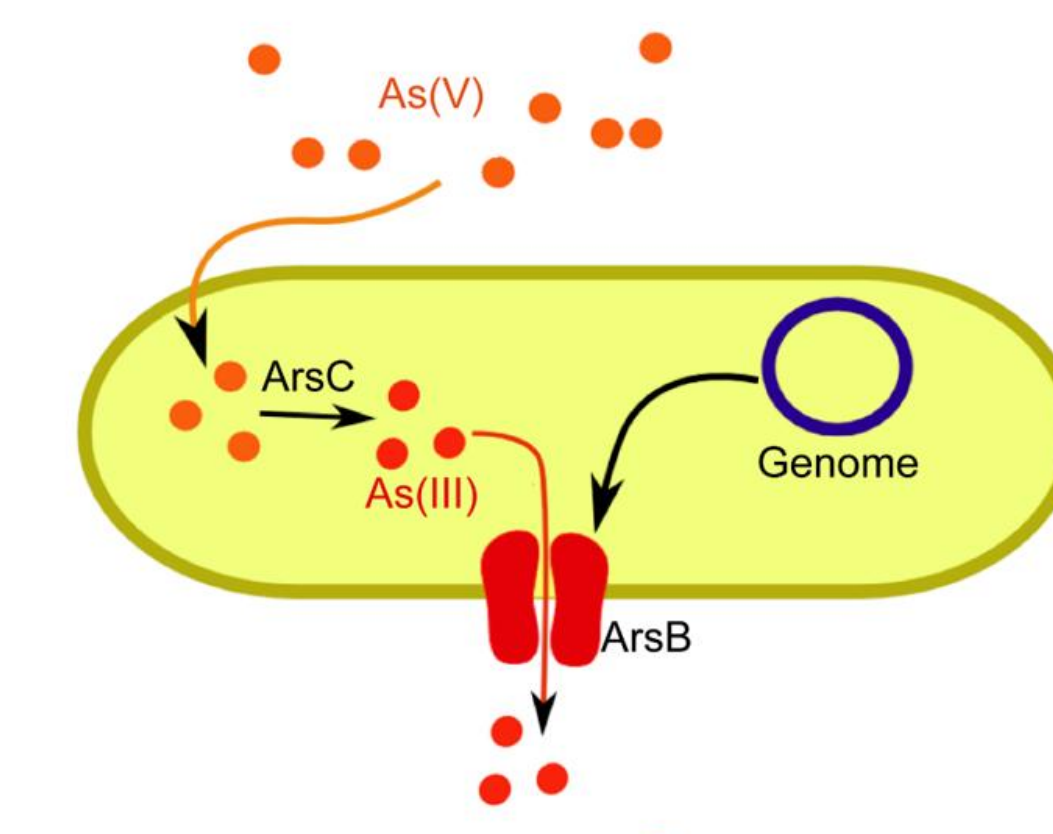
Goals:
- **Trap** arsenic
- **Sequester** it

Approach

Modifying the endogenous arsenic pathway

E. Coli uses the *ArsB* ion transporter to specifically transport arsenite (As(III)) ions out of the cytoplasm. We obtained a single gene knock-out for *ArsB* from the Keio Collection of K-12 derived deletion strains. The ΔArsB strain prevents arsenite efflux.

Phytochelatin and the synthetic Arsenic Binding Peptide sequester arsenite

Metallothioneins are a class of cysteine rich peptides that have been shown to bind heavy metals. Two different approaches were pursued to express metallothioneins in the efflux deficient strain. We introduced a gene into the ΔArsB strain coding for a plant-derived enzyme that catalyzes the formation of phytochelatin from glutathione, which is naturally available in *E. Coli*. Further we adapted a synthetic sequence directly coding for a cysteine rich peptide named the Arsenic Binding Peptide.
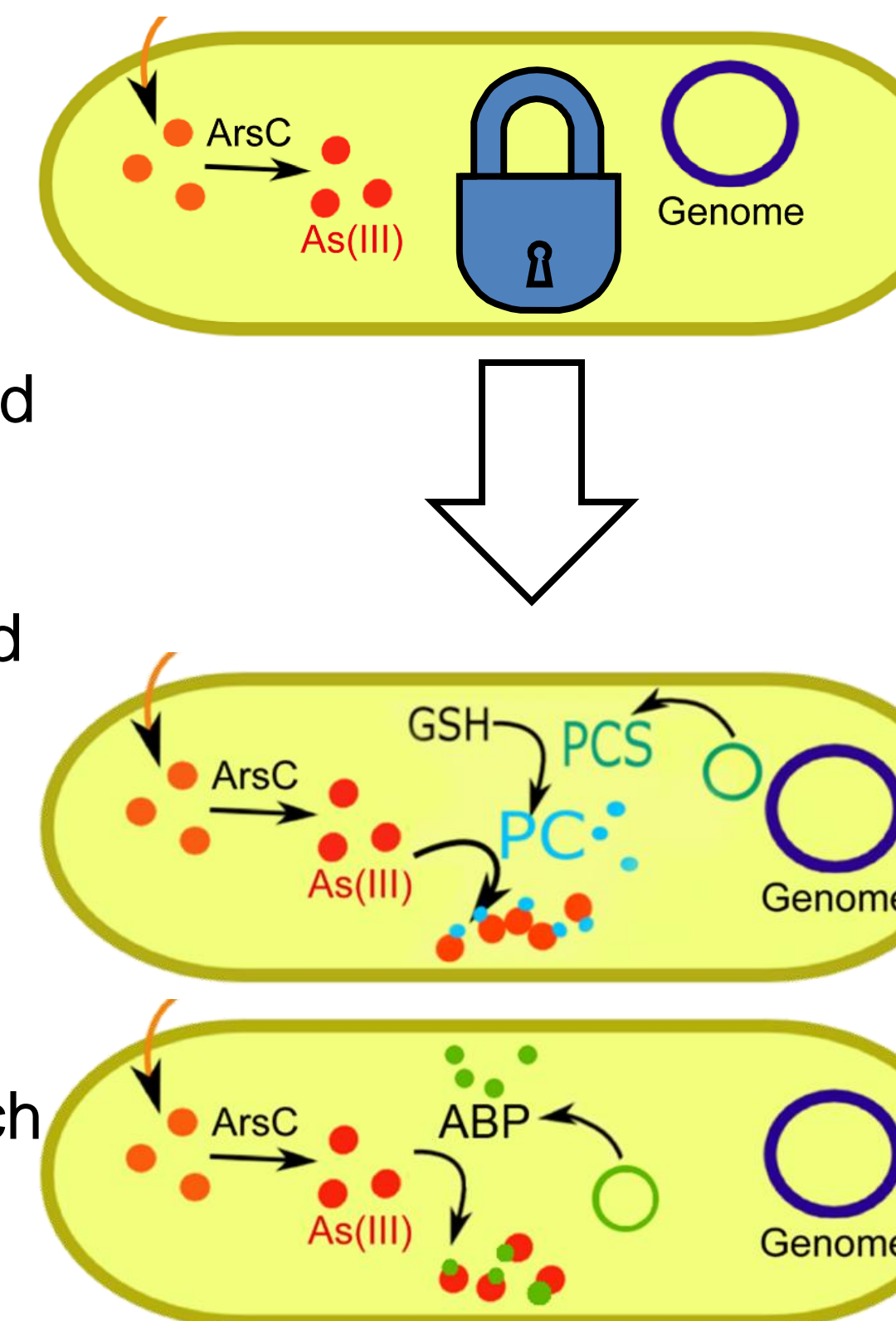
Model

Two compartment model of arsenic pathway

We developed a system of nonlinear ordinary differential equations based on mass balance to describe the concentrations of arsenate (AsV) and arsenite (AsIII). The model is divided into two compartments: intracellular (i) and extracellular (o).

In the graph, extracellular arsenate decays as it diffuses into the cell. Intracellular arsenate initially peaks as the arsenate diffuses into the cell. *ArsC* reduces intracellular arsenate to arsenite at which point intracellular arsenate decays. As intracellular arsenite accumulates the arsenite pump *ArsB* actively transports it out of the cell.

Using HPLC measurements of arsenate and arsenite concentrations, in intracellular or extracellular compartments at various time points, we parameter fit the model to the data using the wild type strain.
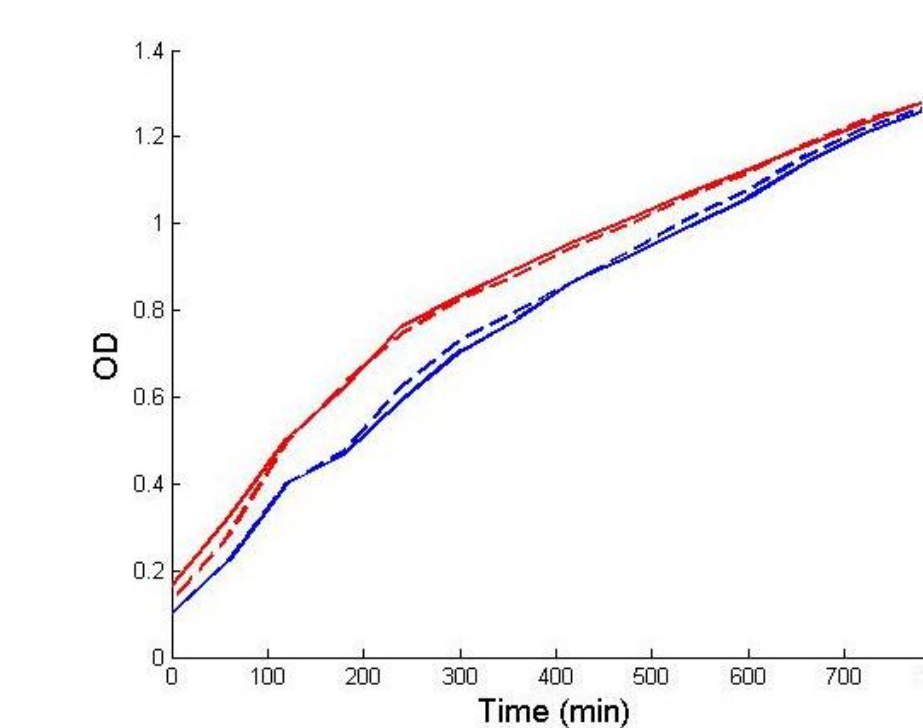
ArsB knockout simulation was then performed with the model, and results were compared to the data obtained from the ArsB knockout strain.
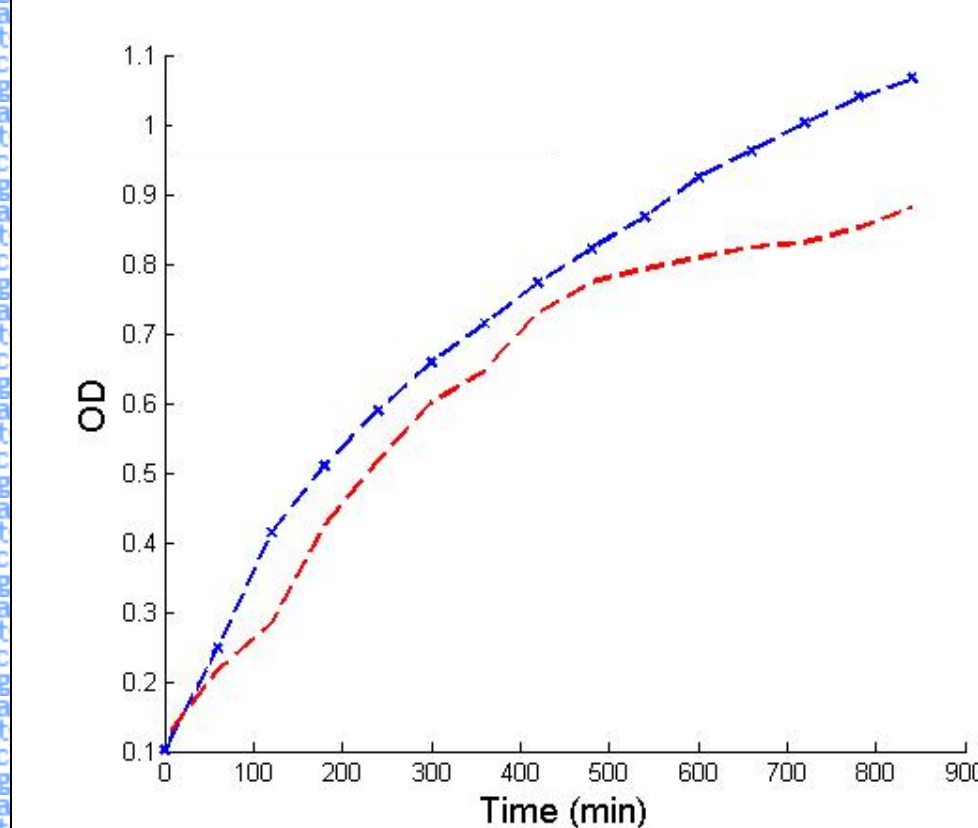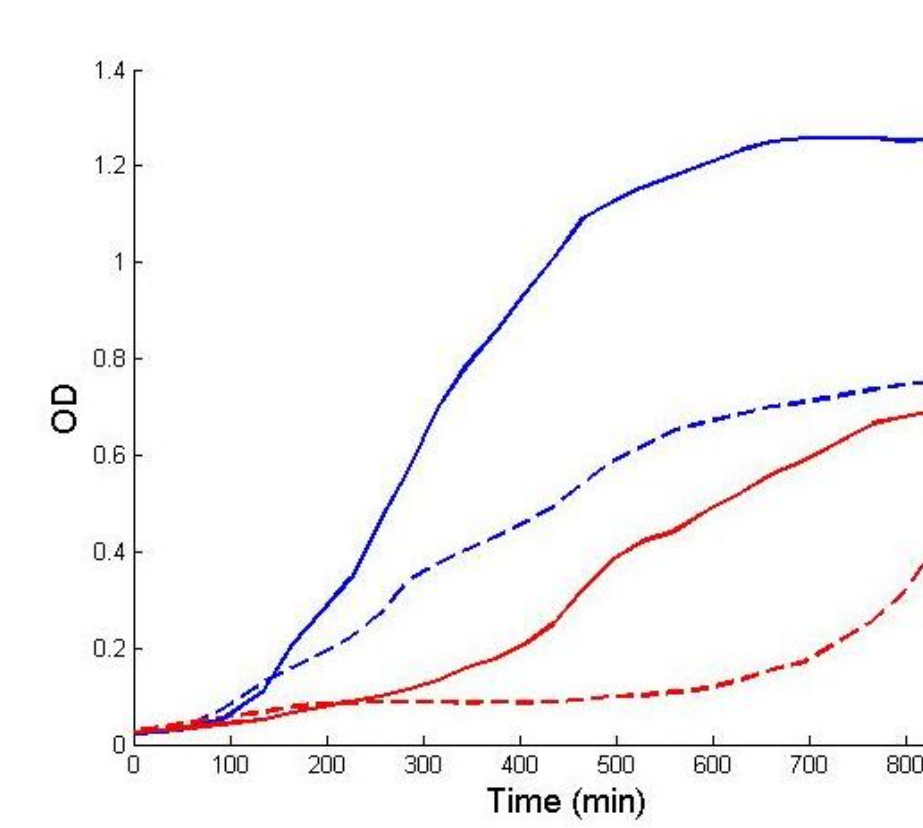
Wild Type Simulation

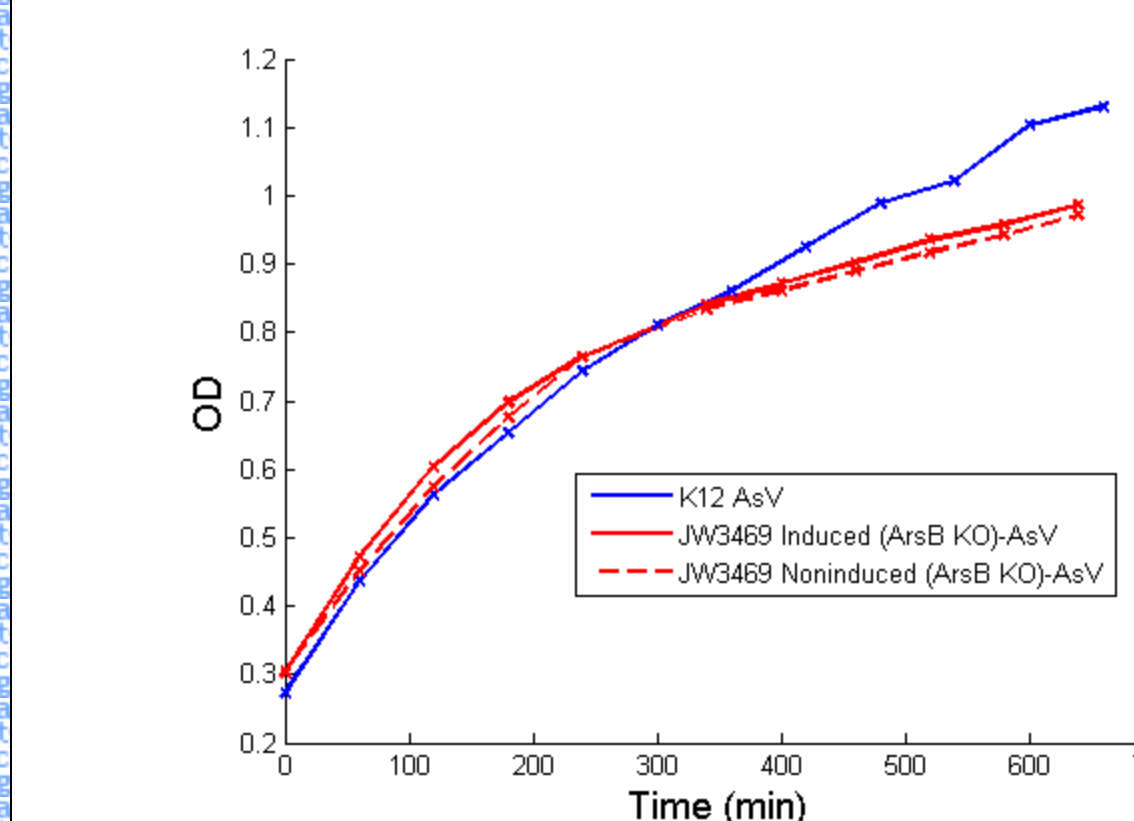**ArsB Knockout Simulation**
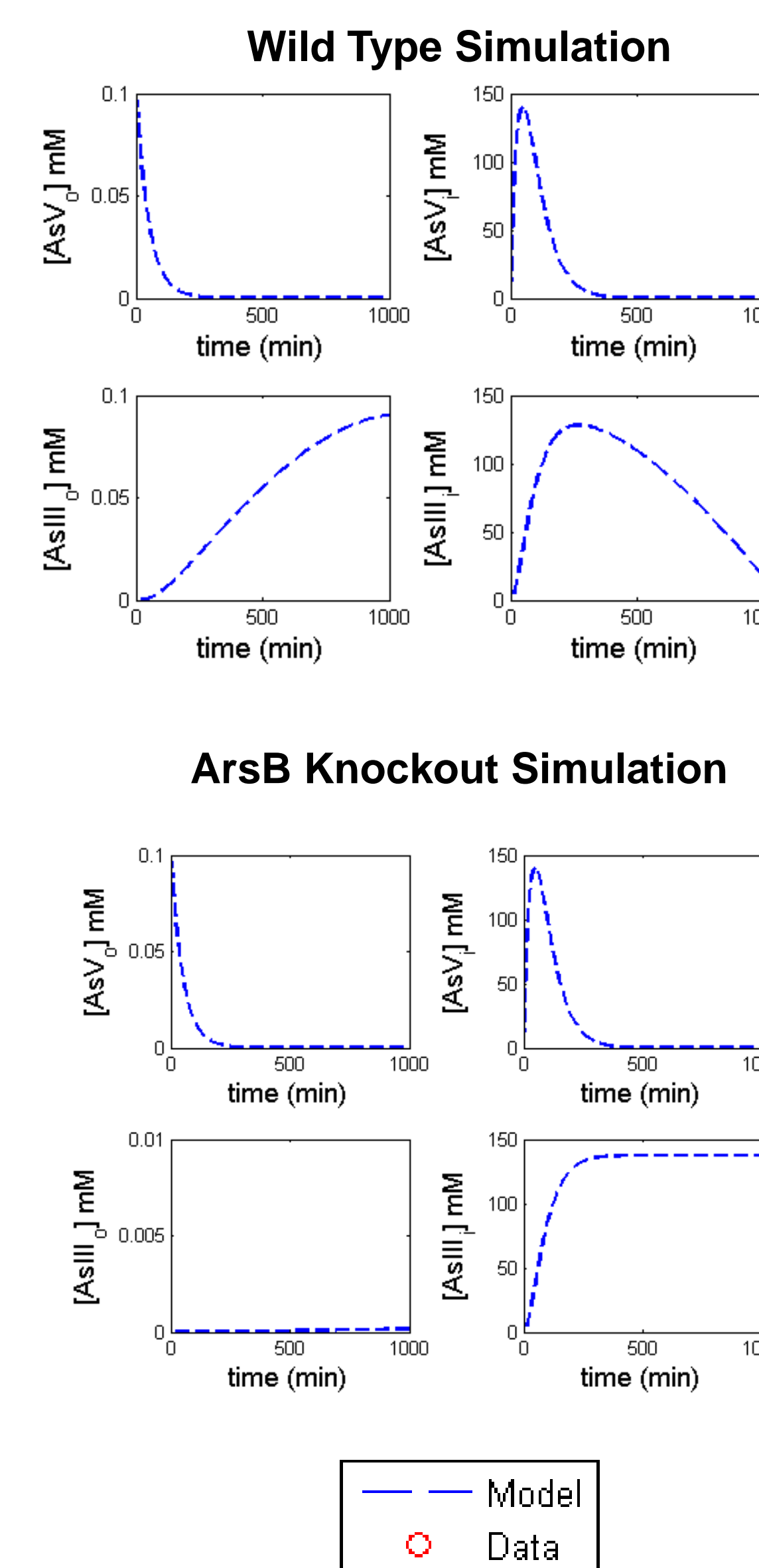
Results
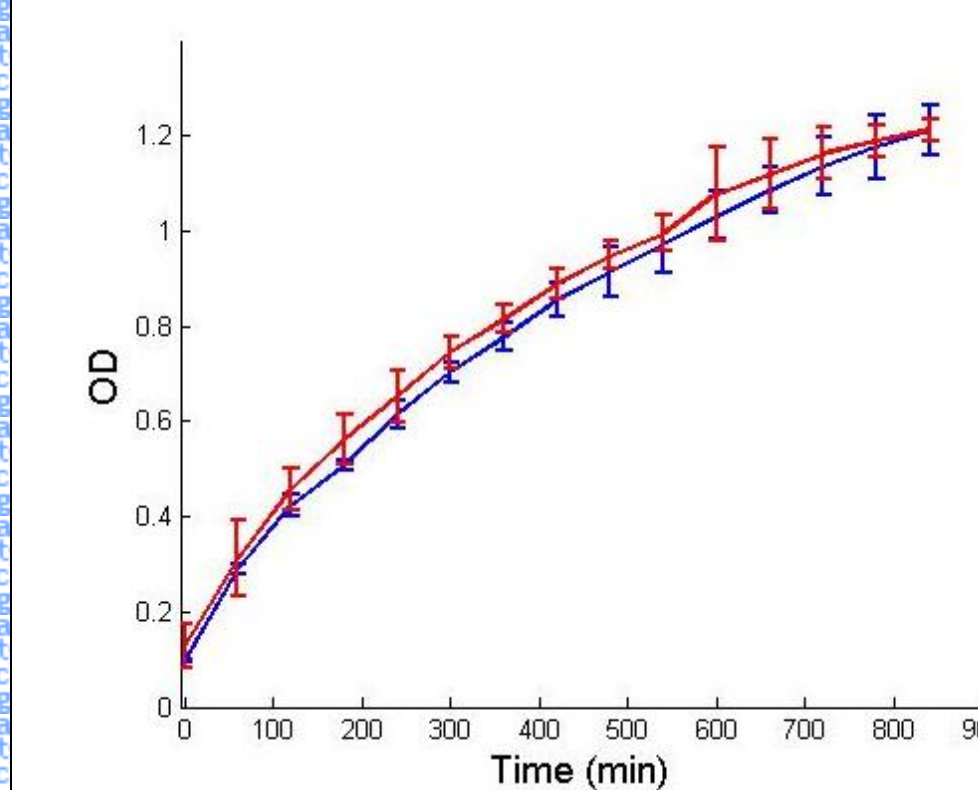
ΔArsB grows comparably to K-12 control

13.3μM arsenic has little effect on growth
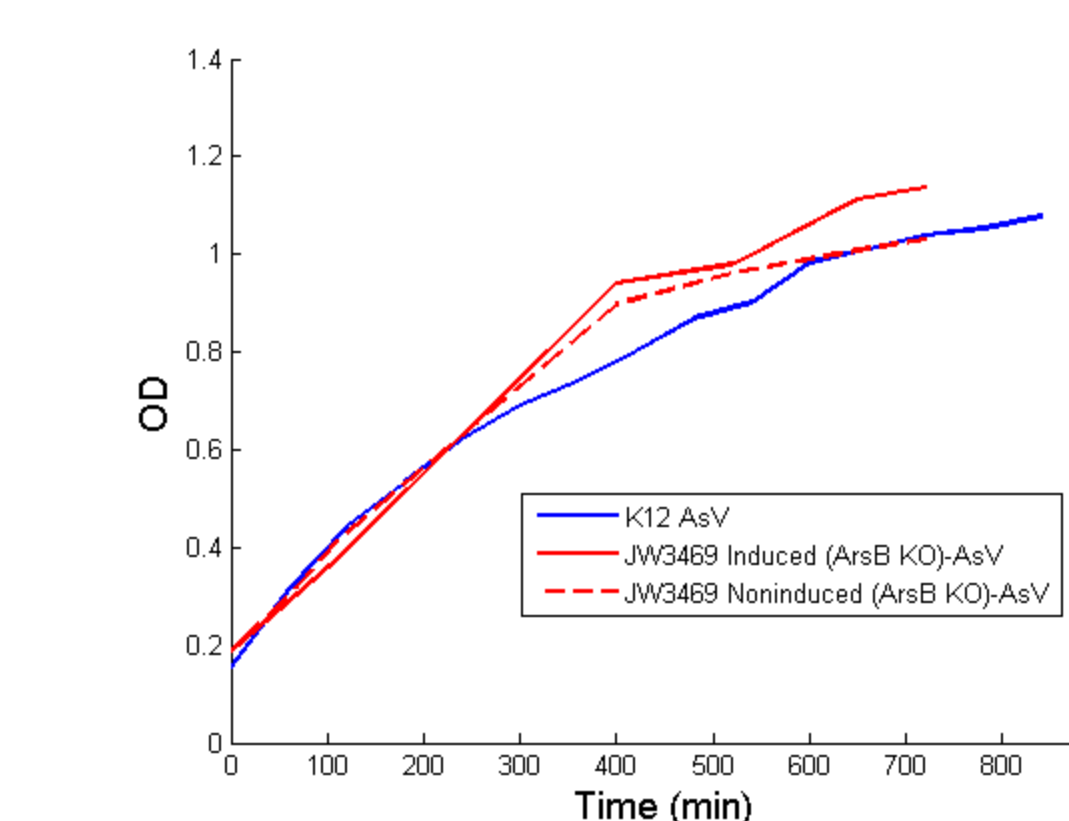
0.1mM arsenic mildly affects ΔArsB growth

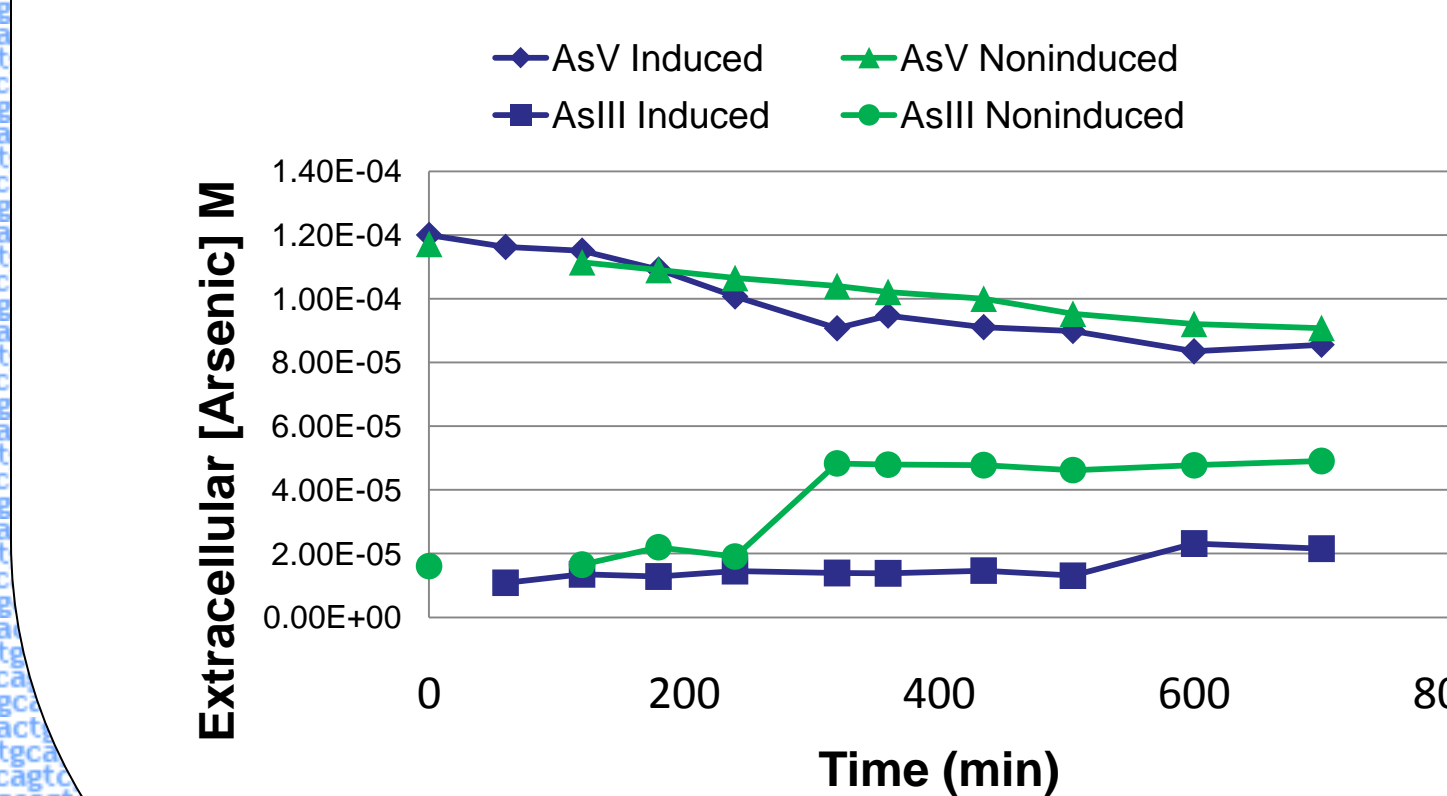2mM arsenic severely affects ΔArsB growth

ΔArsB PCS^{+} induced cells show little difference to controls (0.1 mM AsV)

ΔArsB ABPHA induced cells show some difference to controls (0.1 mM AsV)

However, quantitation using HPLC shows that the engineered strain sequesters more arsenic compared to controls! This supports predictions derived from the model. Notice that while extracellular [AsV] decreased, the extracellular [AsIII] increased slower in the induced sample indicating intracellular sequestration.

Team

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